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Flowcytometric Immunophenotyping Study of Acute Lymphoblastic Leukemia at M.Y. Hospital Indore

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Abstract

Background: *ALL accounts for 12% of all leukemia (but 80% in children). Global incidence is about 3 per 100,000 population, in which 3 out of 4 cases occurring under 6 years age. T-cell acute lymphoblastic leukemia (T-ALL) is a malignant clonal expansion of immature T cells that accounts for 10% to 15% of childhood and 25% of adult ALL cases.*

Objective: *1)To diagnose and subclassify acute lymphoblastic leukemia with the help of flowcytometry and 2) to find out age and sex related incidences of acute lymphoblastic leukemia cases. We emphasizes on the subclassification of ALL as timely and accurate diagnosis of hematologic malignancies is crucial for the appropriate clinical management because ALL responds more for the treatment and the particular subtypes i.e*
B ALL and T ALL requires different therapies, so the subclassification is also important.

Material and Methods: *A one year study was conducted in Pathology department of M.G.M. medical college Indore. Firstly the cases were evaluated with the help of routine peripheral smear and then the relevant cases were evaluated on flow cytometer. We have included 40 cases which were diagnosed as acute leukemia, undifferentiated cells or blasts on peripheral smear.*

Results and Observations: *We observed that majority of the cases they were under 20 year of age. On peripheral smear examination around 37 cases (92.5%) were categorized as acute leukemia. Among acute leukemia cases 34 cases (85%) are of Acute lymphoblastic leukemia. B- acute lymphoblastic leukemia represents most of ALL cases while T- ALL cases are very much rare i.e.33 cases (82.5%) are of B- ALL and 01(2.5%) case of T-ALL.*

Conclusion: *With the help of above thesis work we have observed that ALL is a most frequent childhood hematological neoplasm and the use of Flow cytometry is helpful in diagnosis and identification of ALL subtypes.*

Keywords: *Acute lymphoblastic leukemia, flowcytometry.*

Introduction

ALL is the most common cancer in children. Global incidence is about 3 per 100,000 population, in which 3 out of 4 cases occurring under 6 years age.[(1)] ALL accounts for 12% of all leukemia (but 80% in children).[(2)] T-cell acute

lymphoblastic leukemia (T-ALL) is a malignant clonal expansion of immature T cells that accounts for 10% to 15% of childhood and 25% of adult ALL cases.[3] Multiparameter flowcytometry is a useful technology to find out the morphology and cytochemistry of cells and it is very much helpful in the diagnosis of Acute Leukemia.[4] Flow cytometry is having the capability of detecting a single leukemia cell among 10,000 or more normal cells in peripheral blood during the treatment for newly diagnosed T- lineage ALL in children. [5]

Various CD markers are used for evaluation of leukemias.

Table 1 shows CD markers for different cell lineages.

Lineage	Markers
B lymphoid markers	(CD19, CD10, CD20, CD22, cCD79a)
T lymphoid markers	CD1a,CD2, CD4, CD8) CD3, CD5, CD7,
stem cell/hematopoietic precursors	(CD34, HLA-DR, terminal deoxynucleotidyl transferase/TdT),

Materials and Methods

This study was conducted in Department of Pathology, Mahatma Gandhi Memorial Medical College and M.Y. Hospital, Indore, Madhya Pradesh, India. Approval was obtained from the departmental scientific committee and the institutional ethical committee for the study. The study duration was one year. Sample size for the study was 40 cases. In our study CD markers are used to identify the suspected cases of acute lymphoblastic leukemia and to sub classify further with the help of flow cytometry.

Inclusion criteria: suspected cases of leukemias on peripheral smear.

Cases with acute undifferentiated leukemia

Exclusion Criteria: Already diagnosed cases and Chronic Leukemia cases.

The following CD markers are used - cytoplasmic CD 3 ECD, cytoplasmic 79a PE, CD 45- PC 5 and MPO – FITC. Cytoplasmic CD3 used for T – ALL, 79a PE for B- ALL.

Result and Observation

Table: Age Wise Distribution of Cases

Age Groups	No. Of Cases	Percentage
0 – 15 Years	21	52.5%
15 – 35 Years	09	22.5%
35 – 50 Years	08	20.0%
More Than 50 Years	02	5.0%
Total Cases	40	100%

Cases

Majority of cases they fall under 15 year of age.

SEX	Number of Cases	Percentage
MALE	24	60%
FEMALE	16	40%
TOTAL	40	100%

Table Sex Wise Distribution of Cases

NUMBER OF CASES	ANTI MYELOPEROXIDASE FITC	79 B PE	CYT 3 ECD	CD45 PC5
03	+	-	-	Dim
01	-	-	+	Dim
33	-	+	-	Dim
02	-	-	-	Dim
01	-	-	-	-

Table: Disrubution of cases as per Immunophenotyping

Flow Cytometric Finding	number of Cases	Percentage
B All	33	82.5%
T All	01	2.5%
Aml	03	7.5%
Reactive Lymphocytosis	01	2.5%
Nhl	01	2.5%
Plasmacytoid Lymphoblast	01	2.5%
Total	40	100%

Discussion

In our department this thesis work was carried out over 40 cases during one year duration. We have found that cytoplasmic expression for CD79a was present in every case of B - ALL while T - ALL shows expression for cytoplasmic CD 3, both expresses CD 45 Similar results were found by Zahid Kaleem et al in his study in 2003.Fareed Haddad et al in 2014 also found that around 63% of their patients were children (104 out of 165 patients) with age less than fourteen years old. 114 patients were male while 51 patients were female with male to female ratio 2.2: 1 whereas

Precursor-B- acute lymphoblastic leukemia represents eighty percent (132 patients) of cases which was almost similar to our study.

Some of the study shows that immunophenotypic analysis of acute leukemia by flow cytometry has been used clinically as an important tool for identification of the lineage of leukemic cells and evaluation of the response to treatment [6, 7, 8,9, 10,11]. Recently, the panels of monoclonal antibodies which are specific for lineage-associated antigens have been expanded. As a result, immunophenotyping of ALL has been applied to distinguish it from acute myeloid leukemia

Fareed Haddad et al in 2014 also found that around 63% of their patients were children (104 out of 165 patients) with age less than fourteen years old, which was almost similar to our study.

Kirtika Patel et al studied in 2015 that the ability of the cytometry test to identify correctly those who have ALL (Sensitivity of cytometry test) was 80%. The ability of the cytometry test to identify correctly those who have AML (Sensitivity of cytometry test) was 41.2%.[12] In our study we have also concluded that majority of the ALL cases were diagnosed and subclassified.

T cells that accounts for 10% to 15% of childhood and 25% of adult ALL cases. With wider use of intensive chemotherapy, the prognosis for childhood T-ALL has improved remarkably: nearly 80% of patients can be currently cured.1;2 Further gains in treatment outcome will likely require methods to identify patients who continue to fail on contemporary protocols, so that alternative therapy can be introduced as early as possible.[13]

Conclusion

ALL is more prevalent among children. Subtype B- acute lymphoblastic leukemia represents most of ALL cases while T - ALL cases are very much rare. Different CD markers are available for diagnosis and classification of ALL like cytoplasmic 79a PE expression are for B – ALL and cytoplasmic 3 ECD expression represents T – ALL. Flow cytometry is a better achievement in the field of pediatric oncology, and it will definitively give a better life to the patients and will reduce their side effects.

In general, T-cell ALL has a better prognosis in comparision to mature B-cell ALL. So proper diagnosis plays its role in changing the prognosis of the patient as with wider use of intensive chemotherapy, the prognosis for childhood T-ALL has improved remarkably

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